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TRANSACTIONS

Studies on the assimilation of nitrites by fungi. A Supplement to Part I.

By Kinichiro SAKAGUCHI and Yinchang WANG.

(Agricultural Chemical Laboratory, Tokyo Imp. Univ.)

(Received May 2, 1936.)

The authors, in their previous paper(1), reported that a new medium, which was prepared by substituting $NaNO_2$ (2 g per litre) for $NaNO_3$ of the Czapek's solution, could be conveniently used as a selective or confirmatory medium for certain groups of microorganisms. In the following experiments the authors have tried to apply the new medium for the classification of some black Aspergilli which, according to the previous work(1), exhibited a wide variation in their abilities of the development on the new medium.

EXPERIMENTAL

To determine the most suitable cultural conditions for the differenciation of the moulds by means of the new medium, the agar slants of the following composition are inoculated with the spores of the moulds, which were transferred two times successively at three days' intervals on Kojiagar at 33∼34° C and are of the same age at the time of inoculation, and incubated at various temperatures.

The composition of the medium are as follows.

K_2HPO_4	1.00 g	KCl	0.50 g	$MgSO_4 \cdot 7H_2O$	0.50 g
$FeSO_4 \cdot 7H_2O$	0.01 g	$NaNO_2$	2.00 g	Sucrose	30.00 g
Agaragar	15.00 g	distilled water	1,000 cc.		

The degree of the development of the moulds are expressed as follows.

(+)	The germination of the spores along the streak line is scarcely visible.
+	The development of the mycelium along the streak line is obvious.

++	A feeble growth with scanty conidium-formation.
+++	A moderate growth. The colonies cover the whole surface of the slant.
++++	A vigorous growth with an abundant formation of conidia.
△	The growth is limitted only on the upper portion of the slant.

In the following experiments, three parallel slant cultures were made for each condition. And all the three cultures attained almost uniform growth.

TABLE I.

Aspergillus	Temperature (°C)	The growth after		
		3 days	6 days	10 days
aureus	28～29	+++	++++	++++
	33～34	++	++++	++++
	37～38	++	+++	++++
japonicus	28～29	+++	++++	++++
	33～34	+++	++++	++++
	37～38	++	+++	+++
batatae	28～29	+++	++++	++++
	33～34	+++	++++	++++
	37～38	+++	++++	++++
luchuenis	28～29	(+)	+	++
	33～34	(+)	+ or △	+++
	37～38	(+)	++	+++
ficuum	28～29	(+)	+	△
	33～34	(+)	+	△
	37～38	(+)	+	△
carbonarius	28～29	(+)	+	△
	33～34	(+)	+	△
	37～38	(+)	+	△
pulverulentus	28～29	(+)	(+)	(+)
	33～34	(+)	(+)	(+) or △
	37～38	(+)	(+)	(+)
niger	28～29	(+)	(+)	(+) or △
	33～34	(+)	(+)	(+) or △
	37～38	(+)	(+)	(+)
niger var S.	28～29	(+)	(+)	+ or △
	33～34	(+)	(+)	+
	37～38	(+)	(+)	+ or △

niger var. L	28～29	(+)	+	+
	33～34	(+)	(+)	(+)
	37～38	(+)	+	+ or △
awamori	28～29	(+)	(+)	+
	33～34	(+)	(+)	+
	37～38	(+)	(+)	+ or △
fuscus	28～29	(+)	+	+ or △
	33～34	(+)	(+)	(+)
	37～38	(+)	+	+

Further investigations were made with Asp. luchuensis which gave somewhat divegent results in the above experiment.

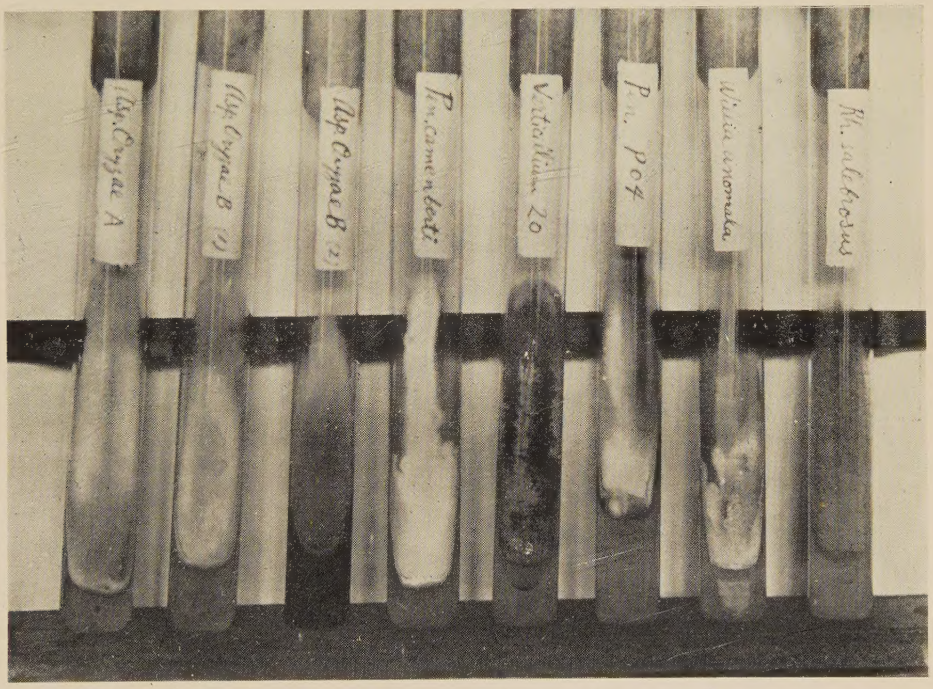

Fig. 1. The growth of various moulds and a yeast upon the new medium. (ref. to Part I, this Journal **11**, 39.)
(Aspergillus; Rhizopus: 33～34°C, 4 days.
Penicillium; Verticillium; Willia: 30°C, 4 days)
Asp. Oryzae B (2) shows the growth upon koji extract agar at the same conditions.

TABLE II

(Investigator)	Sakaguchi and Wang			A. Ozaki, S. Tada and S. Yamazaki	
	(a)	(b)	(c)	(a)	(b)
The growth after 3 days at 33～34°C	+++	++	+++	++	+++

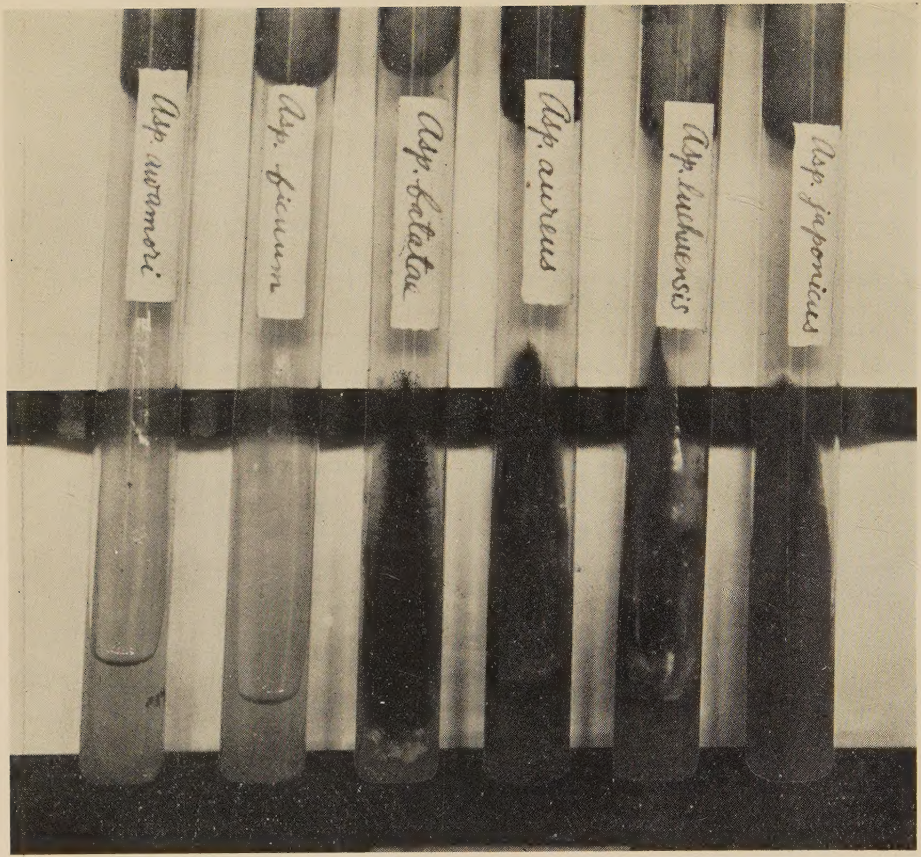

Fig. 2. The growth of black aspergilli upon the new medium at 33∼34°C after 4 days.

SUMMARY

(1) The authors have studied on the application of their nitrite-medium for the classification of black aspergilli.

(2) 2∼5 days' incubation of the agar slant culture at 30∼37°C is sufficient to establish a distinction between the growth rates of the moulds.

(3) The species, which grow luxuriantly on the new medium, are Asp. aureus, Nakazawa ; Asp. japonicus, Saito and Asp. batatae, Saito, while Asp. luchuensis, Inui attains a moderate growth.

Asp. awamori, Nakazawa ; Asp. ficuum, Hennings ; Asp. fuscus ; Asp. niger, Van Tiegem ; Asp. niger var L ; Asp. niger var S ; Asp. pulverulentus, McAlpine and Asp. carbonarius, Bainier give scarcely any sign of growth within the cultural conditions given above.

(4) Black Aspergilli may be divided in two groups (or in three groups) by means of the nitrite-medium.

(5) Asp. luchuensis is distinguished from Asp. awamori by the diffenent adaptability for the new medium.

Studies on the assimilation of nitrites by fungi Part II.

By Kinichiro SAKAGUCHI and Yinchang WANG.

(Agricultural Chemical Laboratory, Tokyo Imp. Univ.)

(Received May 2, 1936.)

In the previous paper(1) the authors have shown that nitrites were able to serve as good nitrogen sources for certain microorganisms especially for moulds, and a new medium, which was prepared by substituting $NaNO_2$ (2 g per litre) for $NaNO_3$ of the Czepek's solution, could be employed not only for maintaining pure cultures but also for determining the identity of some moulds or isolating certain narrow groups. But it was not determined whether nitrites were assimilated directly or after being previously converted to some other nitrogenous compounds, such as nitrates, by certain biological activities of moulds.

In the present work the authors have investigated the nutritive value of the nitrite, as compared with those of the nitrate and the ammonium salt, as well as the mode of assimilation of nitrites by moulds.

EXPERIMENTAL

(1) The preparation of culture media.

The culture media are prepared by adding various amounts of nitrogenous matters to the basal solutions of the definite compositions.

Basal Solution	No. 1	No. 2
distilled Water	1,000 cc	1,000 cc
K_2HPO_4	1.0 g	1.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g	0.5 g
KCl	0.5 g	0.5 g
$FeSO_4 \cdot 7H_2O$	0.01 g	0.01 g
Cane sugar	50.0 g	100.0 g

To 1 litre of the basal solution No. 1 or No. 2 the nitrogen sources are added in the following proportions.

Culture medium	Basal solution	$NaNO_3$	$NaNO_2$	NH_4Cl
a	No. 1	5.10 g	——	——
b	No. 1	——	4.14 g	——
c	No. 1	——	——	3.21 g
ab	No. 1	5.10 g	4.14 g	——

The concentration of each nitrogen source in the above media corresponds to about 0.06 Mol. 300 c.c. of the media are taken into an Erlenmeyer's flask and sterilized, 10 g of sterilized $CaCO_4$ being added subsequently.

The medium bc is prepared by mixing 200 c.c. of the culture medium b′ (6.21 g of $NaNO_2$ in lit. of the basal solution No. 2) and 100 c.c. of c′ (9.63 g of NH_4Cl in 1 lit. of No. 2), both being previously sterilized, with subsequent addition of 10 g of sterilized $CaCO_3$.

(2) The test for the stability of the media.

As nitrites are unstable in the acidic solution and are easily oxidized to nitrates, it is necessary to confirm the stability of the media containing nitrite in the pH-range, in which following experiments are carried out. The following quantities of the salts are dissolved in the Sörensen's phosphate buffer solutions and are kept at 33~34°C. The solutions are analysed at definite intervals.

The solution of $NaNO_2$ and $NaNO_3$.

Periods of incubation (days)	pH-values of the solutions	N_2O_5-N. (mg per 100 c.c.)	N_2O_3-N. (mg per 100 c.c.)	N_2O_5-N./N_2O_3-N.
—	6.37	73.7	73.7	1.00
4	6.65	76.8	75.2	1.02
10	6.80	76.9	77.5	0.98
13	6.51	76.3	75.7	1.00

The solution of $NaNO_2$ and NH_4Cl.

Periods of incubation (days)	pH-values of the solutions	N_2O_3-N. (mg per 100 c.c.)	NH_3-N. (mg per 100 c.c.)	N_2O_5-N.
—	6.36	61.9	79.7	+
4	6.32	60.2	79.5	+
7	6.70	62.0	79.8	+
10	6.48	62.9	79.8	+

As shown in the above tables, no change of the composition is perceived. The concentration of the solutions upon water bath did not as well effect the oxidation of the nitrite.

(3) Methods of analysis.

For the estimation of nitrites in the culture media in presence of nitrates following method is devised. To the neutralized sample an excess of NH_4Cl —about 5 times of the quantity of nitrite (as $NaNO_2$) contained in the sample— is added and kept on boiling upon gentle flame for at least one hour. The nitrite in the sample is thus completely decomposed while the nitrate remains unattacked, which is determined by Schloesing-Wagner's method (Wiley : Principles and practice of agricultural analysis vol. II, p. 397, 1908). To determine the quantity of nitrite, the sample is treated with the solution of K-permanganate and dilute sulphuric acid. Total quantity of nitrate is then determined by Schloesing-Wagner's method. The quantity of nitrite can easily be calculated from the difference between the quantities of nitrate before and after the oxidation. For the detection and estimation of nitrites Griess reagent is also employed.

Ammonia is detected by means of Nessler's reagent and estimated by the distillation with magnesia in vacuo with subsequent titration of the distillate. Sugar is estimated by Bertrand's method after inversion and expressed as sucrose.

(4) Methods for cultivation of moulds.

The stock cultures, which are to be used to inoclate, are transferred two or three times successively at definite intervals (2 days for Asp. Oryzae A, 5 days for Asp. batatae and Asp. aureus) on Koji agar at definite temperatures (Asp. Oryze A at 33～44°C, Asp. batatae and Asp. aureus at 35～37°C), and are of the same age at the time of inoculation.

EXPERIMENT No. 1.

The medium b is inoclated with Asp. Oryzae A. Takakashi and incubated at 33°C. And the formation of nitrate from nitrite is examined.

TABLE I.

Number of flasks	Periods of incubation (days)	Yields of the mould (g.)	pH-values of media after cultivation	N_2O_5–N formed (mg per 100 c.c.)	N_2O_3–N remained (mg per 100 c.c.)	NH_3–N formed (mg per 100 c.c.)	Cane sugar (g per 100 c.c.)
b—0	(original medium)	——	7.49	trace	75.0	——	4.917
b—1	4	0.376	7.02	〃	65.2	——	4.617
b—2	4	0.361	7.98	〃	65.2	——	4.674
b—3	7	2.823	7.09	〃	4.8	0.1	1.207
b—4	7	2.600	6.76	〃	5.9	0.3	1.264
b—5	10	2.996	7.79	〃	trace	0.1	0.192
b—6	10	2.913	7.97	〃	〃	0.2	0.163
b—7	14	2.226	> 8.5	〃	〃	5.6	0.037
b—8	14	2.292	> 8.5	〃	〃	6.2	——

The data of Table I show that the oxidation of nitrite to nitrate does not occur by Asp. Oryzae A, while increasing amounts of ammonia are produced with the periods of incubation. When this mould is cultivated upon the medium, which contains Na-nitrate as its sole nitrogen source, the formation of Na-nitrite is brought about as shown in the following Table.

TABLE II. (medium a)

Number of flasks	Period of incubation (days)	Yields of mould (g.)	pH-values of the media after cult.	N_2O_5-N. remained (mg per 100 c.c.)	N_2O_3-N. formed (mg per 100 c.c.)	NH_3-N. formed (mg per 100 c.c.)	Cane-sugar remained (g per 100 c.c.)
a—0	(original medium)	——	7.71	72.0	trace	——	4.997
a—1	4	0.598	7.02	62.4	0.11	trace	4.679
a—2	4	0.491	7.20	65.8	0.08	〃	4.600
a—3	7	2.544	7.43	30.3	0.01	〃	1.565
a—4	7	2.810	7.33	34.1	0.02	〃	1.577
a—5	10	2.868	7.58	13.4	?	〃	0.216
a—6	10	2.800	7.75	12.5	〃	〃	0.131

The results shown in table I and II seems to lead to a conclusion that by Asp. Oryzae A nitrite is assimilated directly while nitrate may perhaps be taken after being previously reduced to the nitrite⁽²⁾.

TABLE III. The selective assimilation test for Na-nitrite and Na-nitrate by Asp. Oryzae A. (medium a b)

Number of flasks	Peuiods of incubation (days)	Yields of the mould (g.)	pH-values of the medium after cult.	N_2O_5-N. remained (mg per 100 c.c.)	N_2O_3-N. remained (mg per 100 c.c.)	NH_3-N. formed (mg per 100 c.c.)	Cane sugar remained (g per 100 c.c.
ab—0	(original medium)	——	7.58	73.4	74.9	——	9.690
ab—1	4	0.428	7.24	67.5	71.7	——	9.576
ab—2	7	0.763	6.67	73.0	55.0	0·3	7.600
ab—3	7	0.695	6.78	70.5	49.4	0.3	7.190
ab—4	10	2.978	6.81	65.8	trace	0.2	3.624
ab—5	10	2.891	6.84	70.3	〃	1.2	3.073
ab—6	14	4.854	7.84	38.0	——	5.6	0.121
ab—7	14	3.700	6.79	71.6	trace	trace	2.009

From the results of the above experiment, it is evident that when Asp. Oryzae A is cultivated upon the medium, containing Na-nitrite and Na-nitrate as its nitrogen sources (the medium ab), the mould assimilates the nitrite selectively. The nitrate is attacked only after the complete consumption of the former, as shown in the case of ab-6 in the above table.

TABLE IV. The assimilation of ammonium salt (NH_4Cl).

Number of flasks	Periods of incubation (days)	Yieds of the mould (g)	pH-values of the medium after cult.	N_2O_5-N. formed (mg per 100 c.c.)	N_2O_3-N. formed (mg per 100 c.c.)	NH_3-N. remained (mg per 100 c.c.)	Cane sugar remained (g per 100 c.c.)
c—0	(original)	——	7.33	——	trace	74.8	4.934
c—1	4	0.504	6.33	——	〃	67.0	4.655

c—2	4	0.277	6.33	——	〃	71.3	4.698
c—3	7	2.337	6.83	——	〃	14.0	1.477
c—4	7	1.960	6.62	——	〃	23.6	1.807
c—5	10	3.721	7.16	——	〃	trace	0.250
c—6	10	3.806	7.21	——	——	〃	0.175
c—7	14	3.217	7.43	——	——	4.2	0.061

TABLE V. The selective assimilation test for Na-nitrite and NH_4Cl.

Number of flasks	Periods of incubation (days)	Yields of the mould (g)	pH-values of the medium after cult.	N_2O_5-N. formed (mg per 100 c.c.)	N_2O_3-N. remained (mg per 100 c.c.)	NH_3-N. remained (mg per 100 c.c.)	Caue sugar remained (g per 100 c.c.)
bc—0	(original medium)	——	7.33	trace	72.8	79.1	9.854
bc—1	4	0.176	6.55	0.6	68.1	72.8	9.386
bc—2	4	0.285	6.48	0.9	70.6	71.5	9.329
bc—3	7	1.569	6.86	4.2	36.6	44.5	4.921
bc—4	7	1.069	6.85	3.7	49.9	51.4	6.753
bc—5	10	4.996	7.12	11.4	6.6	19.0	0.290
bc—6	10	3.144	7.20	6.4	trace	35.0	1.857
bc—7	14	4.464	8.02	3.5	〃	29.7	0.036

Table V illustrates that the nitrite and the ammonium salt are consumed nearly at the same rate, althoush the former disappears earlier in the medium. It is very interesting that, in spite of no nitrate could be found from the Na-nitrite and the ammonium chloride when they are given separately (see table I and IV), relatively large amounts of the nitrite are produced when both nitrogen sources are present in the same medium.

EXPERIMENT No. 2.

In the following experiments Asp. batatae, Saito and Asp. aureus, Nakazawa are used instead of Asp. Oryzae A in the experiment No. 1. Among black Aspergilli which, according to our previous work$^{(3)}$, do not generally grow well on our nitrite medium, the both species belong to few members which are able to attain fairly luxuriant growth.

TABLE V.

Culture media	Periods of incubation (days)	Yields of the mould (g)	pH-values of the medium after cult.	N_2O_5-N. formed or remained	N_2O_3-N. formed or remained	NH_3-N. formed or remained	Caue-sugar remaining (g per 100 c.c.)
a	(original medium)	——	7.73	71.0	trace	——	4.973
b	〃	——	7.72	trace	75.7	——	4.950
ab	〃	——	7.54	73.9	75.7	——	9.358
c	〃	——	7.64	——	trace	——	4.608
bc	〃	——	7.20	trace	73.9	——	9.541

Asp. batatae	a	8	4.584	7.54	35.7	——	trace	0.490
	b	8	0.904	7.54	5.7	42.8	〃	2.708
	ab	8	4.342	7.66	74.2	21.2	〃	3.259
	c	13	4.496	5.64	——	——	13.7	——
	bc	13	0.454	6.37	6.0	53.9	57.9	——
Asp. aureus	a	10	1.199	6.55	58.4	——	trace	3.624
	b	10	0.667	7.07	3.2	65.0	〃	3.624
	ab	10	2.507	6.74	79.5	18.2	——	5.331

The results of Table VI illustrate that the mode of assimilation of nitrite by those black species seems to be fairly different from that of Asp. Oryzae A. While no nitrate was formed from Na-nitrite by Asp. Oryze A, appreciable quantities of the former are produced from the latter by the black species. When both salts are present in the same medium (ab) the nitrite is assimilated selectively. In the medium bc, which contains ammonium salt and nitrite, the results similar to those of Asp. Oryzae A were obtained.

SUMMARY.

(1) A method for the estimation of nitrite in presence of nitrates is devised.

(2) In the medium, which contains natrium nitrate as its sole nitrogen source, the nitrite is formed by Asp. Oryzae A, Takahashi.

(3) The Oxidation of the Na-nitrite to the nitrate is effected by Asp. aureus and Asp. batatae, but not by Asp. Oryze A.

(4) Neither nitrate nor nitrite is produced from the ammonium chloride by Asp. Oryzae A.

(5) The nitrite is assimilated selectively by all three species in presence of the nitrate, which is attacked only after the complete consumption of the former.

(6) The nitrite and the ammonium salt are consumed nearly at the same rate, although the former disappears somewhat earlier in the medium.

(7) Small quantities of ammonia are always formed in old cultures.

(8) Generally speaking, the nitrite may be regarded as an intermediate substance in the resorption of the nitrate by the moulds, especially by Asp. Oryzae A. The degree of the oxidation of the nitrite to the nitrate seems to vary in reference to the nature of the moulds.

The authors wish to express their heartiest thanks to Professor Dr. T. Takahashi, for encouragement given them in the present work.

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(3) See the preceeding paper, (a supplement to part I, Sakaguchi and Wang).

ABSTRACTS
from
TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

On the free Hexonbases and the free Sugars of Iridaea laminarioides. (pp. 421 ~ 424): By Tetutaro TADOKORO and Chokkan TAKASHUGI. (Faculty of Agriculture, Hokkaido Imperial University, Sapporo, Japan.)

Untersuchungen von Essigbakterien aus Formosa. (IX). (s. 425 ~ 442): Von Syoosuke TANAKA. (The Department of Industry, Government Research Institute, Taiwan Japan.)

On the unsaponifiable matter of Chrysalis oil. (pp. 425 ~ 456): By Jun-ichi OZAKI and Bunzo KASAI. (The Tokyo Imperial Sericultural College).

The unsaponifiable matter prepared from the oil of the chrysalis of the silk worm moth, Bombyx mori, has been studied by different workers. The results of their investigations, however, are contradictory, especially as far as the sterols are concerned.

The author found that the Chrysalis oil contained 2.93 per cent unsaponifiable matter which consisted of 18.07 per cent hydrocarbon and 81.93 per cent sterol.

The constituents of the unsaponifiable matter were studied chiefly by the fractional crystallization with several kinds of organic solvents and isolated the following substances.

1. Hydrocarbons.

Four hydrocarbons were separated. These did not absorb Bromine in ether solution and melted at 62.5°, 56.0°, 50 ~ 50.5° and 46.5° respectively. Besides these properties, by the elementary analysis and the determination of molecular weight, the author assigned to each hydrocarbon the following formula.

mp.	62.5°	$C_{28}H_{58}$
mp.	56.0°	$C_{26}H_{54}$
mp.	50~50.5°	$C_{24}H_{50}$
mp.	46.5°	$C_{22}H_{46}$

2. Sterols.

Two kinds of sterols, A and B, were isolated.

A Sterol. From its alcoholic solution this sterol crystallised with one molecule of water in laminae which were discerned under the microscope as very thin plates, frequently showing re-entering angles. Melted at 147°. The rotation in chloroform solution: $[\alpha]_D^{20} = -38.23$.

Acetate: Needles. mp. 114~115°. Acetyl value 129.21.
$[\alpha]_D^{20} = -41.69$.

Acetate dibromide: mp. 108~109°. Bromine contents 27.16%.

From the above analysis, the author concluded that this sterol was identical with cholesterol ($C_{27}H_{46}O$).

B Sterol. From alcohol this sterol crystallised in the form of plates or solid needles with one molecule of water, but from ether as anhydrous needles.

mp. 139~140; $[\alpha]_D^{20} = -34.27$

Sterol dibromide: Soluble in a mixture of ether and glacial acetic acid and appeared to crystallise with difficulty. Blackend at 102~103°.

Acetate: Plates. mp. 128~128.5°.
Acetyl value 130.63. $[\alpha]_D^{20} = -40.56$

Acetate dibromide: Long needles. mp. 113~114°.
Bromine contents 27.27%

Benzoate: Rectangular plates. mp. 145°.
Mol. Wt. 481.44

All the above mentioned properties are very similar to those of ordinary sitosterol.

These results have led the author to conclude that the sterols consisted of a mixture of cholesterol and sitosterol.

Studies on the Nodule Bacteria. IX.—On the electrical properties of the accessory substance. (pp. 457~466): By Arao ITANO and Akira MATSUURA. (Ōhara Institute for Agricultural Research, Kurashiki, Japan.)

In the previous paper, it was reported that the stimulation of the growth of nodule bacteria is due to the organic substances which are soluble in water, alcohol and chloroform especially 80～90 percent of it is extracted by the combined action of water and alcohol while the inorganic substances have no action. In this investigation, an enquirely was made to ascertain the electrical nature of accessory substance.

The extracts of bean nodules were prepared in water, hot water, alcohol and hot water, and dialysed. At the sametime, the yeast extract was dialysed and experimented also.

The results of experiments are summarised as follows:

(1) By the electro-dialysis, it was not possible to eliminate completely the accessory substance in the bean nodule but a partial elimination was possible.

(2) The accessory substance was mainly found in the cathodic chamber or the substance itself was anodic while none was found in the anodic chamber.

(3) Morphologically the short rod forms were numerous while the bacteroids were very few. Comparatively more bacteroids were present in the cathodic content.

(4) The yeast extract was very inactive in the electro-dialysis cells, and it was not possible to obtain the comparative data with the bean nodule.

(5) No relationship was found between the accessory substance and the nitrogen content but the hydrogen ion concentration seems to have influence on the accessory substance. The pH value at the anode was less than 3.0, and at the cathode, 8.0 while it was about 3.5 in the middle chamber.

Studies on the Amylase of Yeast. (IX).—On the enzymic chemical Properties. (6). Properties of the Yeast-Amylase on Glycogen. (pp. 467～474): By Kazuki OHNO. (Biochemical Laboratory of Taihoku Imperial University, Japan.)

Studies on the Principal Constituents of Tobacco (I).—On the Difference of Nicotine Contents of the Tobacco Leaves of the different Parts. (pp. 475∼496): By Shinji KANAHARA. (Japanese Government Monopoly Bureau.)

Über Grayanotoxine, die wirksamen Inhaltsstoffe von *Leucothoe grayana*, **II. Mitteil.**(1) (S. 497∼502): Von Sikiro MIYAJIMA und Sankiti TAKEI. (Aus d. Agriculturchem. Institut d. Universität Kyoto 2. 1939).

Wie in einer früeren Mitteilung(1) berichtet wurde, haben wir ausser Methyläther-p-cumarsäure drei wirksame Inhaltsstoffe aus Blättern von *Leucothoe grayana* krystallinisch gewonnen. Grayanotoxin-I (Schmp. 249°) und -III (Schmp. 210∼260°; nicht näher bestimmt) liefern beide durch Pyridin-Acetylierung dasselbe Acetylderivat von Schmp. 226° (*Triacetylgrayanotoxin-I* bzw. *Tetraacetylgrayanotoxin-III*). Beim Alkaliverseifen geht das *scharf* schmelzend Tetraacetylderivat in das *unscharf* schmelzende Grayanotoxin-III zurück, welches 1/2 Mol. Krystall-Lösungsmittel enthält, wie z. B. $C_{20}H_{34}O_6 \cdot 1/2H_2O$ und $C_{20}H_{34}O_6 \cdot 1/2CH_3CO_2C_2H_5$. Werden Grayanotoxin-I und -III mit Essigsäureanhydrid und Essigsauresnatrium acetyliert, ergeben sie keine krystallinischen Produckte, wenn man aber die amorphen Acetylprodukte mit Alkali verselft, so liefern beide denselben Stoff von Schmp. 220∼224°, der die Formel $C_{20}H_{32}O_5$ besitzt und *Anhydrograyanotoxin-III* ist. Anhydrograyanotoxin-III ist ein Isomer von Grayanotoxin-II (Schmp. 198°), und beide enthalten eine leicht mit Brom nachweisbare Doppeldung. Bei Pyridin-Acetylieren ergibt Grayanotoxin-II ein Tetraacetylprodukt von Schmp. 172° und geht durch katalytische Hydrierung in *Dihydro-tetraacetylgrayanotoxin-II* (Schmp. 170°) über, das auch aus Dihydrograyanotoxin.II (Schmp. 246∼260°) durch Pyridin-Acetylierung gewinnbar ist.

Nach unseren Erfahrungen enthalten nur die Frühlingblätter die oben genannten drei Grayanotoxine, in Sommerblättern fehlt das Grayanotoxin-I und schlechte getrocknete Sommerblätter sowie auch Äste liefern nur Grayanotoxin-II, aber kein Grayanotoxin-I oder -III. *Es liegt die Annahme nahe, dass Grayanotoxin-I der primär in der Pflanze gebildeter Stoff ist der in Grayanotoxin-III und fernerhin Grayanotoxin-II sich umwandelt.*

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(1) I. Mitteil.: J. Agr. Chem. Soc. Japan; 10 (1934), 1093.

Studies on "Kaoling" as the Source of Starch-Making, Milling and "Ame"-Manufacture (IV).—Part I. Some preliminary Experiments for woshing, Steeping and Cooking of recoater Kaoliang. (pp. 503∼511): By R. YAMAMOTO, S. YOSHIMURA & R. TANAKA. (Central Laboratory, South Manchurrian Railway Co.)

Chemical Studies on Japanese Coccidae. XI.—On the resinous constituents of Ceroplastes rubens. 1. (pp. 512∼520): By M. KAWANO and R. MARUYAMA. (Laboratory of Ohsaka Factory of Sankyo Co. Ltd.)
